969-114 Novel Ultrasonic Contrast Agent Targeted to D-Dimer Using DD-3B6 Monoclonal F(ab) in Vitro

Gregory M. Lanza, Kirk D. Wallace, Paul R. Eisenberg, Michael J. Scott, William P. Cacheris, Donald H. Christy, Angela M. Sharkey, James G. Miller, Samuel A. Wickline. *Jewish And Barnes Hospitals at Washington University, St. Louis, MO*

To facilitate sensitive and specific detection of vascular thromboses, we have recently developed a targetable ultrasonic contrast agent (nongaseous, biotinylated lipid encapsulated perfluorocarbon emulsion, mean particle size 250 nm) that permits noninvasive detection of fibrin clots by binding to molecular ligands associated with thrombi and enhancing acoustic reflectivity. The targeted ultrasonic contrast agent was prepared by biotinylating the fluorocarbon emulsion particles. To mimic small, suspended, crosslinked fibrin thrombi, D-dimer was coated directly onto 0.8 μ polystyrene beads and ultrasonically imaged with a 7.5 MHz linear phased array before and after exposure to the ligand-targeted contrast agent. The polystyrene particles appeared opaque within dialysis tubing but manifested little ultrasonic backscatter. Sequential addition of a biotinylated monoclonal $F_{(ab)}$ fragment directed against the D-dimer epitope 3B6, followed by avidin, followed by biotinylated acoustic contrast rapidly increased the echogenicity of the suspension. To mimic surface adherent, crosslinked fibrin thrombus, D-dimer was covalently bound to nitrocellulose membranes and ultrasonic imaging was performed before and after targeting with the perfluorocarbon emulsion with a high resolution, broadband 50 MHz acoustic microscope. The targeted ultrasonic contrast increased the backscatter of the nitrocellulose membranes at all frequencies between 30 and 60 MHz, by an average 4.6 ± 0.1 dB ($p < 0.05$) above controls (2.9 fold enhancement). These results demonstrate the potential of this novel, site-targeted perfluorocarbon ultrasonic contrast agent to specifically bind to molecular epitopes in fibrin clots, enhance their acoustic reflectivity, and suggest a clinical role for clot detection with the use of ultrasound.

969-115 Is Five-Year Mortality Different for Treatment by Choice vs. Random Assignment in the Bypass Angioplasty Revascularization Investigation (BARI)?

Katherine Detre, Allan Rosen, Robert Jones, William Rogers, Michael Mock, David Faxon, Martial Bourassa, Frederick Feit, Michael Cowley, Floyd Loop and the BARI Investigators. *University of Pittsburgh, Pittsburgh, PA*

BARI recruited 4110 patients with multivessel coronary artery disease requiring revascularization who were suitable for either CABG or PTCA. Consent for random treatment assignment was obtained from 1829 patients; another 2013 preserved the right to chose their treatment but agreed to be followed in a registry, and 268 refused to participate. Within 3 months of study entry, CABG was the initial procedure for 32% of registry patients, PTCA for 58%, and the remaining 10% were maintained on medication only.

	Tx by Random Assignment	Tx by Choice
Age ≥ 65	39%	40%
Female	27%	26%
African American	6%	4%
Post HS Education	29%	40%
Prox/Mid LAD	76%	69%
# Lesion > 50%	3.1	3.0
% Myocardium Jeopardized	61.1	59.2
Hx MI	55%	51%
Hx CHF	9%	5%
Unstable Angina	64%	61%

The 5-year mortality for patients in the BARI trial will be compared to the BARI registry adjusting for differences in patient characteristics between the groups.

970 Molecular and Genetic Determinants of Coronary Artery Disease and LVH

Tuesday, March 26, 1996, Noon–2:00 p.m.
Orange County Convention Center, Hall E
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970-116 New Genetic Risk Factors for Coronary Artery Disease

Marisa Serrato, Qun-Tao Yu, Faye Safavi, Robert Roberts, Ali J. Marian. *Baylor College of Medicine, Houston, Texas*

Coronary artery disease (CAD) is a complex trait caused by a number of genetic and environmental factors. Techniques of molecular genetics provide the opportunity to identify genes that may confer increased susceptibility for CAD. Human paraoxonase/arylesterase (HUMPONA), involved in oxidation of LDL cholesterol, β-fibrinogen, necessary for thrombosis, and the renin angiotensin system (angiotensinogen (AGT), angiotensin II receptor I (ATIIR1a)), which induces cell proliferation, are all involved in atherosclerosis. Variants of these genes are known to encode for proteins that enhance their respective function and are, thus, potential candidate genes for altered susceptibility to atherosclerosis. Thus, we determined the frequency of these variants in a genetically homogenous population with angiographically documented CAD and in a comparable group in the general population. DNA was extracted from the blood and genotypes were determined by polymerase chain reaction, gel electrophoresis and/or restriction mapping. The two variants of AGT (M235T, T174M) and that of ATIIR1a exhibited a similar frequency in both groups, while that of HUMPONA (G92A) and β-fibrinogen (G/A^{-455}) showed increased frequency in the group with CAD as shown in the Table.

Genotypes	Control	CAD	Odds Ratio	P value
HUMPONA (G92A)				
A/A	120 (49%)	68 (30%)	2.2 (1.5–3.1)	<0.0001
A/G + G/G	127 (51%)	155 (70%)		
β Fibrinogen (G/A^{-455})				
G/G	153 (54%)	135 (75%)	2.5 (1.7–3.8)	<0.0001
G/A + A/A	130 (46%)	46 (25%)		

The protein product of the HUMPONA gene variant is known to increase the rate of oxidation of LDL cholesterol several-fold and the β-fibrinogen variant is known to be associated with increased plasma levels of fibrinogen, both of which provide a rationale for increased risk of CAD and coronary thrombosis. Identification and characterization of nonconventional genetic risk factors for CAD will provide not only insight into the pathogenesis of atherosclerosis but also risk stratification for improved prevention and treatment.

970-117 Circulating VCAM-1 Correlates With the Extent of Human Atherosclerosis, in Contrast to Circulating ICAM-1, E-selectin and Thrombomudulin

Karlheinz Peter, Peter Nawroth, Wolfram Schief, Thomas Nordt, Thomas Weiss, Christoph Bode. *University of Heidelberg, Germany*

Secondary prevention of atherosclerosis, prior to causing clinical symptoms, could be possible with a serum marker for atherosclerosis. Circulating, shedded forms of adhesion molecules may serve as such, since the cell surface expression of adhesion molecules is upregulated on endothelial cells covering atherosclerotic plaques. In 52 patients with peripheral arterial vascular disease the extent of atherosclerosis was evaluated based on angiograms of a large portion of the arterial system (the abdominal aorta, the pelvic and leg arteries). The area diseased by atherosclerosis was determined by the percentage of vessel wall irregularities of the calculated segments (total: 250 cm^2).

The serum concentration of circulating VCAM-1 demonstrates a strong correlation to the extent of atherosclerosis ($r = 0.8$, $p < 0.001$, $y = 7.3x$ ng/ml cm^{-2} + 294 ng/ml), in contrast to circulating ICAM-1, E-selectin, or thrombomodulin (as a marker for endothelial cell damage). The angiographically evaluated patients were divided in a group with minor ($n = 30$, area < 90 cm^2) and a group with major atherosclerotic changes ($n = 22$, area > 90 cm^2). The serum level of circulating VCAM-1 allows to separate these two stages of atheroscleroses with a high statistical significance (623 ± 157 ng/ml versus 1238 ± 528 ng/ml, $p < 0.001$).

Conclusions: Circulating VCAM-1 demonstrates a strong correlation to the extent of human atherosclerosis. Whether patients who will profit from agressive diagnosis and treatment can be identified by increased levels of circulating VCAM-1 remains to be proven.

970-118 Presence and Distribution of Vascular Wall Adrenomedullin in Experimental Atherosclerosis

Eric E. Williamson, Michihisa Jougasaki, Iftikhar Kullo, John C. Burnett Jr.. *Mayo Clinic, Rochester, MN*

Adrenomedullin (ADM) is a newly discovered potent vasorelaxant peptide, recently demonstrated to be synthesized by cultured endothelial and vascular smooth muscle cells. Although ADM may function as an autocrine and paracrine factor in cardiovascular homeostasis, its activity in disease states such as atherosclerosis has not been defined. The present study was performed to determine the presence and distribution of ADM in normal and atherosclerotic rabbit aorta by immunohistochemistry utilizing a highly specific antibody to rabbit ADM. Studies were designed to test the hypothesis that ADM is present in normal aortic endothelial and vascular smooth muscle cells and is increased in atherosclerosis in response to vascular wall injury. To test this hypothesis, aortic tissue was obtained from normal ($n = 4$) and cholesterol fed rabbits ($n = 14$) (1% cholesterol diet for 8–10 weeks). In normal control

rabbits, intense bands of positive immunostaining for ADM was observed in the endothelial and immediate subendothelial regions, with minimal staining in underlying vascular smooth muscle. In contrast, the atherosclerotic aortas demonstrated positive immunostaining in the vascular smooth muscle as well as in the atherosclerotic plaque, with a loss of endothelial staining in damaged endothelium. The current study demonstrates for the first time the presence of immunoreactive ADM in the normal endothelium. Secondly, the studies also demonstrate the loss of endothelial ADM immunoreactivity in atherosclerosis with markedly increased activity in the atherosclerotic plaque and underlying vascular smooth muscle in hypercholesterolemic atherosclerosis. These studies support an important role for ADM in cardiovascular regulation and its potential activation in experimental atherosclerosis.

970-119 Polymorphisms in the Angiotensin I-Converting Enzyme and Apolipoprotein E Genes and Late Luminal Narrowing After Coronary Angioplasty in Men

Christian Moussard, Alain Vuillemenot, François Schiele, Nicolas Meneveau, Jean-Claude Henry, Jean-Pierre Bassand. *University Hospital Besançon, FRANCE*

Polymorphisms in the angiotensin I-converting enzyme gene (ACE I/D) and apolipoprotein E (apo E) may be linked to coronary heart disease, with respectively alleles D and e4 as risk factor. Therefore we investigated the relationship between the ACE I/D and apo E genotypes and the incidence of restenosis after coronary angioplasty. *Methods.* Amplification of genomic DNA using polymerase chain reaction (PCR) and restriction enzyme fragment length polymorphisms (RFLP) yielded the ACE genotypes II, ID and DD, and Apo E genotypes (e2e3, e3e3, e2e4, e3e4 and e4e4) in 91 male patients with CAD. *Results.* The frequency of the DD genotype was significantly higher among CAD patients than controls (38/91 = 0.42 *versus* 35/122 = 0.29, $p = 0.05$). No significant difference in DD genotype frequency was found between patients with restenosis (defined as > 50% diameter stenosis at 6 months follow-up) and patients without restenosis (18/37 = 0.49 *versus* 20/54 = 0.37, $p = 0.27$). Nevertheless D allele relative frequency was higher in patients with restenosis (0.72 *versus* 0.59, $P = 0.09$). The mean difference in minimal coronary lumen diameter between post-PTCA and follow-up angiogram (late loss) was no significantly different in the 3 groups of ACE genotype. The relative e4 allele frequency was higher among CAD patients than controls (0.16 *versus* 0.10, $p = 0.05$). The distributions of apo E genotypes or alleles in restenosis group was not significantly different from that of the non-restenosis group. Late loss was similar in patient with or without allele e4 in their genotype. *In conclusion,* Polymorphism in the angiotensin I-converting enzyme and apolipoprotein E genes had no influence on the occurence of restenosis after coronary angioplasty.

970-120 D Polymorphism of the Angiotensin Converting Enzyme Gene Is Strongly Associated With the Development of Physiological Left Ventricular Hypertrophy

Hugh Montgomery, Peter Clarkson, Clare Dollery, Krishna Prasad, Maria-Angela Losi, Deborah Statters, Mick Jubb[1], Jean McEwan, Stephen Humphries, William McKenna. *UCL and St George's Medical Schools, London, UK;*[1] *RAMC Millbank, London, UK*

The I/D polymorphism of the angiotensin-converting enzyme (ACE) gene comprises the presence (insertion allele, I) or absence (deletion allele, D) of a 297 base pair fragment. The D allele is associated with higher circulating (and possibly tissue) ACE levels. We tested the hypothesis that renin-angiotensin systems control myocardial growth, by examining the influence of ACE genotype on physiological left ventricular hypertrophy. *Method and Results:* ACE genotype was determined in 134 male British Army recruits. B-natiuretic peptide(BNP) levels (a hormone whose levels are linked to LV mass) and echocardiographically-determined posterior wall(PW) and septal thickness (ST) and left ventricular mass(LVM) were measured at the start and end of 10 weeks of intensive physical training. All measures of LV hypertrophy were associated strongly with the D allele (table shows changes in all parameters: p for heterogeneity < 0.001: < 0.08†)

	II	ID	DD
ST (mm)	−0.03	0.08	0.14*
PW (mm)	−0.03	0.08	0.13*
LVM (g)	−1.27	47.2	56.9*
LVM/height (g/cm)	−0.01	0.27	0.32*

BNP levels rose by 40%, 64% and 180% respectively ($p < 0.01$).

Conclusions: Left ventricular growth in response to exercise is strongly determined by ACE genotype. This finding has important implications concerning the role of renin-angiotensin systems in pathophysiological LVH.

970-121 Serum Autoantibodies Against G-Protein Coupled Receptors in Myocardial Diseases

Shinobu Matsui, Michael Fu[1], Tomoyuki Hirakawa, Kohei Teraoka, Mitsuru Hayase, Noboru Takekoshi, Eiji Murakami. *Kanazawa Medical University, Japan;*[1] *Göteborg University, Sweden*

There is an increasing evidence for the participation of cellular and humoral autoimmunity in the pathogenesis of various myocardial diseases. The purpose of this study was to assess the pathophysiological role of autoantibodies against G-protein coupled receptors in myocarditis(MC), dilated(DCM) and hypertrophic(HCM) cardiomyopathy. Five peptides corresponding to the sequences of the second extracellular loops of the human $\beta 1$-, $\beta 2$, $\alpha 1$, muscarinic cholinergic(M2) and angiotensin-II(AII) receptors were used as antigens in an enzyme immunoassay to screen sera from patients with MC(N = 18), DCM(n = 28), HCM(n = 23) and the age-matched healthy blood donor (HBD)(n = 23).

[Results] Detection of autoantibodies by ELISA.

	$\beta 1$	$\beta 2$	$\alpha 1$	M2	AII
DCM(n = 28)	9 (32%)**	2 (7%)	4 (14%)	10 (36%)*	1 (4%)
MC (n = 18)	6 (33%)**	2 (11%)	3 (17%)	2 (11%)	3 (17%)
HCM(n = 23)	3 (13%)	1 (4%)	2 (8%)	5 (22%)	0 (0%)
HBD(n = 23)	2 (9%)	2 (9%)	2 (9%)	3 (13%)	3 (13%)

**$p < 0.05$, *$p < 0.1$ vs. HBD

[Conclusion] A subgroup of patients with DCM and myocarditis have autoantibodies specifically directed against the second extracellular loops of the $\beta 1$ and/or M2 receptors. These autoantibodies (anti-$\beta 1$ and anti M2) could play an important role in pathogenesis of myocarditis and DCM. These findings also indicate that the evolution of DCM after myocarditis might be predominantly due to an autoimmune mechanism.

970-122 Increased Frequency of the Deletion Allele of the ACE Gene in African-Americans Compared to Caucasians

Baxter D. Montgomery, Elizabeth A. Putnam, John Reveille, Dianna M. Milewicz. *The University of Texas Health Science Center at Houston, TX*

The insertion/deletion (I/D) DNA polymorphism of the angiotensin converting enzyme (ACE) gene has been studied in patients with heart disease. The DD genotype has been found to be associated with increased left ventricular mass (LVM) independent of systolic or diastolic blood pressure (BP). African-Americans have more left ventricular hypertrophy (LVH) than Caucasians with equivalent BP. The D allele has been found at a frequency of approximately 50% in studies of Caucasian populations. We compared the frequency of the D allele in the above populations. We hypothesize that the frequency of the D allele will be increased among African-Americans(AA) as it may explain a genetic contribution to the increase frequency of LVH among African-Americans. *Methods:* Genomic DNA was harvested from peripheral blood leukocytes obtained from 29 unrelated, healthy African-Americans and 29 Caucasians. The genotype of the ACE gene was determined by PCR using the genomic DNA samples and standard primers and conditions.

Results:

Populations	I allele (%)	D allele (%)	p value
AA	15 (26%)	43 (74%)	<0.05
Caucasians	27 (47%)	31 (52%)	

Conclusions: The frequency of the D allele is significantly increased in the population of African-Americans compared to Caucasians. These data suggest that a possible genetic contribution to the increased incidence of LVH found in the population of African-Americans.

971 Outcomes Assessment in Coronary Artery Disease

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Orange County Convention Center, Hall E
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971-77 Cost Avoidance Through Early Discharge of the Uncomplicated Acute Myocardial Infarction Patient

Eric L. Eisenstein, L. Kristin Newby, J. David Knight, Leslee J. Shaw, Robert M. Califf, Eric J. Topol, Daniel B. Mark. *Duke University Medical Center, Durham, NC*

Analysis of GUSTO-I data has shown that uncomplicated acute myocardial infarction (UCMI) patients (without reinfarction, ischemia, stroke, shock, heart